Large Phase Differences between L-Cone– and M-Cone–Driven Electroretinograms in Retinitis Pigmentosa

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Purpose. To study the dynamics and interactions of the signals originating in the long- (L-) and middle (M)-wavelength–sensitive cone pathways in patients with retinitis pigmentosa (RP).

Methods. Twenty-six patients with RP and 29 normal subjects participated in the study. Electroretinographic (ERG) responses were measured to stimuli that modulated exclusively the L- or the M-cones or the two simultaneously (both in-phase and in counter-phase) with varying ratios of L- to M-cone contrasts. S-cones were not modulated.

Results. The data of the normal subjects and of the patients can be described by a model in which the amplitudes and the phases of the signals originating in the L- and M-cones are vector summed. In the RP patients, there was a general reduction in ERG sensitivity. The L-cone–driven ERG response was significantly delayed, whereas the M-cone–driven ERG response was phase advanced.

Conclusions. Large dynamic differences between L- and M-cone–driven ERGs can be detected in RP. As a result, the interaction between the L- and M-cone systems, when modulated simultaneously at 30 Hz, is subtractive in RP patients and additive in normal subjects. Our data show that the use of only a standard white flicker ERG might lead to a misinterpretation of the mechanisms involved in retinal disorders, because the phases of different cone-driven responses are not considered. (*Invest Ophthalmol Vis Sci.* 2000;41:3225–3233)

The term retinitis pigmentosa (RP) is used for a group of progressive retinal diseases representing the most frequent retinal dystrophy with a prevalence of approximately 1:4000 and a frequency of heterozygotes of approximately 1:15.[1–3] Approximately 1.5 million people are affected around the world.[4] Detailed studies over the past years have shown that this disorder consists of genetically and clinically heterogeneous subtypes with different modes of genetic transmission and different types of progression.[5–7]

It has been shown that in early cases of RP the scotopic (rod) electroretinogram (ERG) is markedly reduced, whereas the photopic (cone) flash ERG is relatively normal.[8] The cone-driven responses to 30-Hz white flicker have normal or reduced amplitudes, and they are usually delayed.[4,9–12] To date, it is unresolved how changes in the different cones or their postreceptoral pathways contribute to these delays.

There have been attempts to differentiate between the involvement of the three different cone systems in RP. RP patients exhibited reduced short (S-) wavelength–sensitive cone-driven ERGs; a subset of those patients showed significantly greater loss in the S-cone–driven ERG than in the mixed long (L)- and middle (M)-cone–driven ERG,[13] suggesting that at least the S-cone and the L-/M-cone systems are differently affected. However, it is not known whether the L- and M-cone systems are also affected differently. The purpose of this study was to examine how the L- and M-cones and their postreceptoral systems are affected by RP.

We measured ERG responses to stimuli that either selectively modulated the L- or the M-cones or modulated the two simultaneously. Extensive data on normal subjects have been published recently.[14] RP patients showed generally larger ERG thresholds for nearly all combinations of L- and M-cone modulation. Surprisingly, the L-cone–driven ERG was very much delayed, whereas the M-cone–driven ERG was phase-advanced compared with normal observers. RP patients showed smaller ERG thresholds to counter-phase modulation than to in-phase modulation of the L- and M-cones, indicative of a subtractive interaction between the L- and M-cone–driven ERGs. This is probably caused by the increase in phase difference between the L- and M-cone–driven responses. (The term "L- and M-cone–driven ERGs" is used to refer to the responses originating in the L- and the M-cones, including the subsequent postreceptoral stages. The uncertainty about the exact cellular origins of the ERG does not influence the data interpretation.)

Methods

Subjects

Twenty-six patients (age range, 11–59 years) with different forms of RP (7 autosomal dominant RP, 1 autosomal recessive RP, 3 Usher II syndrome, 10 simplex RP, 1 X-linked RP [X-RP], and 4 carriers of X-RP) participated in the study. The diagnosis was based on history, symmetrical bilateral involvement, the typical alterations of the pigment epithelium layer, by visual field, and Ganzfeld electroretinography according to the ISCEV standard,[15] including the standard 30-Hz flicker ERG (30-Hz-fERG). Twenty-nine normal subjects (age range, 9–57 years) served as controls.

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Informed consent was obtained from all subjects after explanation of the purpose and possible consequences of the study. This study was conducted in accordance with the tenets of the Declaration of Helsinki and with the approval of our institutional ethical committee in human experimentation.

Visual Stimulation and ERG Recording

The method of visual stimulation and ERG recording has been described previously.[14,16] Briefly, the stimuli were presented on a computer-controlled monitor (BARCO CCID 121) driven at 100 Hz by a VSG 2/3 graphics card (Cambridge Research System). The spectral characteristics of the monitor phosphors were measured with a spectroradiometer (Instrument Systems). The luminance output was calibrated using the internal luminance measuring device of the BARCO monitor. The VSG software automatically performed the gamma correction. The monitor subtended 124 by 108° at the 10-cm viewing distance. We used 30-Hz square wave modulation of the red, green, and blue phosphors with predefined Michelson contrasts. The time-averaged luminance of the monitor was 66 candela (cd)/m^2 (40 cd/m^2 for the green phosphor, 20 cd/m^2 for the red phosphor, and 6 cd/m^2 for the blue phosphor). The time-averaged chromaticity in CIE (1964) large field coordinates were $x = 0.3329$ and $y = 0.3181$. The excitations in all cone types by the monitor phosphors were calculated by multiplying the phosphor emission spectra with the psychophysically based fundamentals.[17] The modulation of cone excitation was quantified by the Michelson cone contrast and defined stimulus strength for each cone type separately. The S-cones were not modulated (i.e., the S-cone contrast was 0% in all conditions). In the majority of normal subjects, we measured ERG responses to 32 different stimuli: Eight conditions of different L- to M-cone contrast ratios (1:1; −1:1; 1:2; 0:1; 2:1; −2:1; −1:2: 1:0; negative ratios indicate counter-phase modulation) with four contrasts at each condition (100%, 75%, 50%, and 25% of the maximally possible cone contrast). In the RP patients, we limited the number of measurements to the four most important conditions of L- to M-cone contrast ratios (1:1, 1:0, 0:1, and −1:1), which allowed the simultaneous measurements of reliable amplitudes and of response phases of cone-driven ERGs. The different conditions were presented in a quasi-random order. Owing to the broad emission spectra of the blue and green phosphors, the possible cone contrasts were limited, see Fig. 1 in Usui et al.[16] The maximal cone contrast in the L-cone–isolating condition (M- and S-cones were both silently substituted, i.e., their contrasts were 0%) was 24.7% and 31.2% for the M-cone–isolating condition (double silent substitution for L- and S-cones).

We assumed that the cone photopigment absorption spectra in RP patients were identical with those of the controls. However, a decrease in photopigment density would cause a change in the absorption spectra and, thus, in cone contrasts. Our calculations have shown that halving the density in all cone types would result in only a moderate change in cone contrasts.

ERG recordings were obtained from one eye for all subjects. The pupils of the normal subjects were dilated with 0.5% tropicamide, those of the patients with both 0.5% tropicamide and 5% phenylephrine. The eyes were kept light-adapted for at least 10 minutes before the ERG recording. Corneal ERG responses were measured with DTL fiber electrodes (UniMed Electrode Supplies), which were positioned on the conjunctiva directly beneath the cornea and attached with the two ends at the lateral and nasal canthus. The reference and skin electrodes (gold cup electrodes) were attached to the ipsilateral temple and the forehead, respectively. The signals were amplified and filtered between 1 and 300 Hz (Grass Instruments) and sampled at 1000 Hz with a National Instruments AT-MIO-16DE-10 data acquisition card. ERG responses to 12 runs, each lasting 4 seconds, were averaged in each measurement.

Results

ERG Responses and Model Fits

Figure 1 shows the ERG responses to in-phase modulation of the L- and the M-cones (L–M cone contrast ratio, 1:1), to pure L-cone modulation (L–M cone contrast ratio, 1:0), to pure M-cone modulation (L–M cone contrast ratio, 0:1), and to counter-phase modulation of the two cone types (L–M cone contrast ratio, 1:−1) at similar cone contrasts for a normal subject (Fig. 1A) and a patient with a dominant form of RP (Fig. 1B). Several aspects of these original ERG tracings are of interest. In the normal observer, the responses to in-phase modulation of the L- and the M-cones are larger than to counter-phase modulation and to the cone-isolating stimuli, which cannot be explained by the slightly larger cone contrast in the in-phase condition. This suggests that the responses originating in the L- and M-cones interact additively. The RP patient displays equal amplitudes for the L-cone–isolating condition and only slightly reduced amplitudes for the M-cone–isolating condition. In contrast to the ERGs of the normal observer, the response amplitude to counter-phase modulation is larger than to in-phase modulation despite the smaller cone contrast. This suggests subtractive interactions between the L-cone– and the M-cone–driven ERG responses.

The ERG responses were Fourier analyzed, and the ERG response amplitude and phase were defined as the amplitude and phase of the fundamental component. We found an approximately linear relationship between ERG response amplitude and cone contrast for all conditions in the RP patients and the normal subjects. This is shown in Figure 2 for two stimulus conditions: The in-phase and the counter-phase modulation of the L- and M-cones (cone contrast ratios 1:1 and 1:−1, respectively).

The slope of the linear regression to the data is the increase in ERG amplitude per percent increase in cone contrast. This slope was used to define the cone contrast gain. In analogy with the contrast gain that is used to define the sensitivity of single neurons in the visual system (e.g., see Ref. 18), the ERG contrast gain quantifies the ERG sensitivity. As can be seen in Figure 2, the slope of the linear regression in the in-phase condition is larger than the one in the counter-phase condition for normals. The reverse is true for the RP patients. This indicates that the L- and M-cone–driven responses are additive in the normals and subtractive in the RP patients.

The inverse of the cone contrast gain is the cone contrast increase needed for a 1-μV response increase,[14] which, owing to the linear relationship between amplitude and cone contrast, is equivalent to a threshold. The cone contrast gains and the thresholds were obtained for all ratios of L- to M-cone contrasts.

Figure 3A shows the measured ERG thresholds for six normal subjects. The ellipses are fits of a model, based on the assumption that the ERG responses are the results of a vector summation of the ERG signals originating in the L- and M-cones.

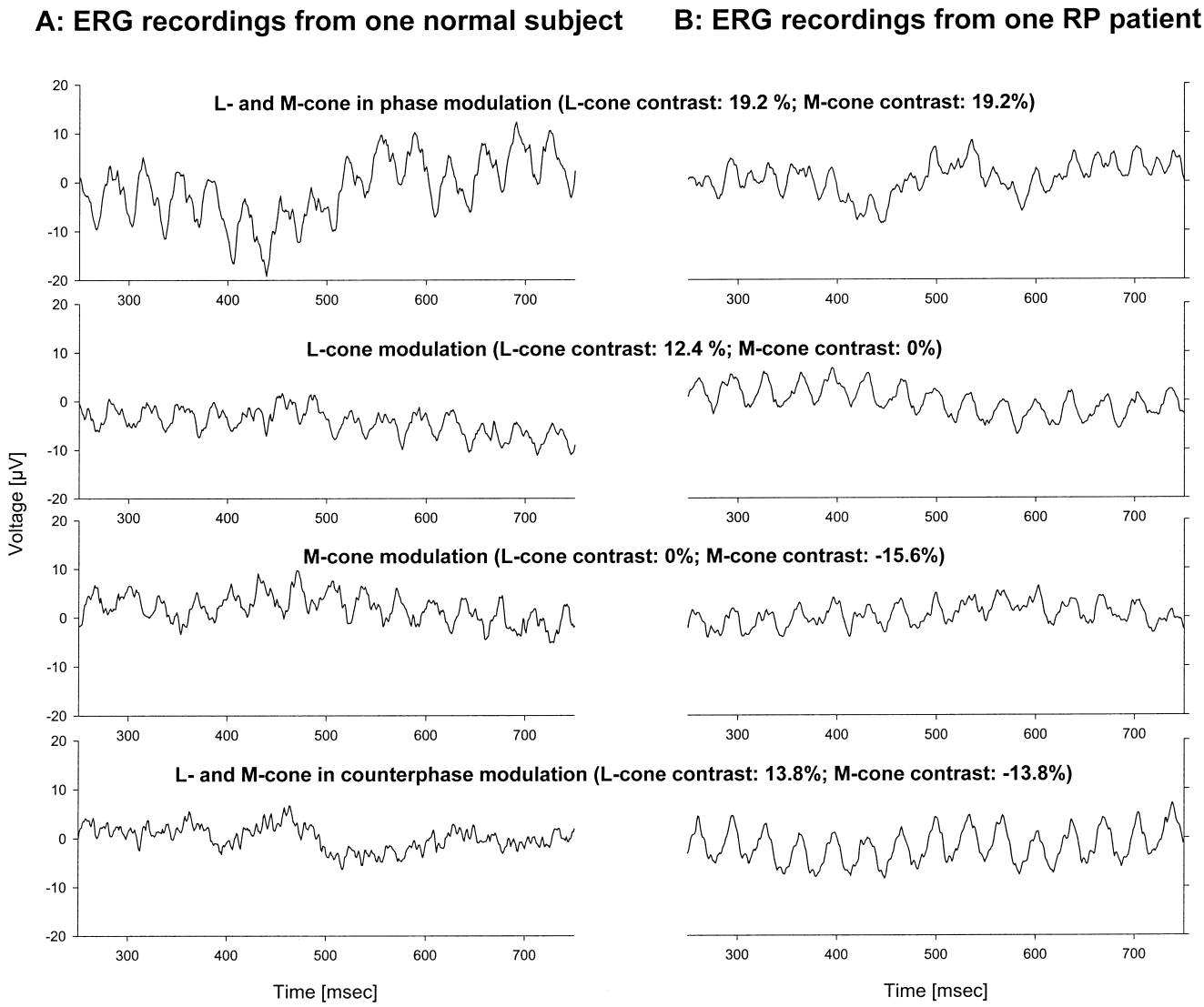

FIGURE 1. Averaged ERG responses to in-phase modulation of the L- and M-cones (*upper row*: L–M cone contrast ratio 1:1; 19.2% L-cone contrast; 19.2% M-cone contrast), to pure L-cone modulation (*second row*: L–M cone contrast ratio 1:0; 12.4% L-cone contrast; 0% M-cone contrast), to pure M-cone modulation (*third row*: L–M cone contrast ratio 0:1; 0% L-cone contrast; −15.6% M-cone contrast), and to counter-phase modulation of the two cone types (*lower row*: L–M cone contrast ratio 1:−1; 13.8% L-cone contrast; −13.8% M-cone contrast) for a normal subject (**A**, *left*) and a patient with a dominant form of RP (**B**, *right*). The ERG signals are 500-msec extracts out of 4-second traces that are the averages of 12 runs. Positive and negative cone contrasts indicate in-phase and counter-phase modulation with the red monitor phosphor, respectively, which was used to synchronize the stimulus with the data acquisition.

A detailed description of the model can be found elsewhere.[14] Briefly, we assume that the signals originating in the L- and the M-cones have separate weightings (defined by the cone contrast gains) and phases and that the total response is simply the addition of the two separate responses at each instant. Because the responses are basically sinusoidal without intrusion of higher harmonics (see also Ref. 19), they can be expressed as vectors, the lengths of which are determined by the amplitudes; the angles with the positive *x*-axis are equivalent to the phases. As a result of the above-mentioned assumption, the response vector to a combination of L- and M-cone modulation is equal to the vector addition of the two response vectors with cone-isolating conditions. In the fits of this model to the threshold data, there are three free parameters: the L-cone weighting or L-cone contrast gain (A_L), the M-cone weighting or the M-cone contrast gain (A_M), and the phase difference between the L-cone– and the M-cone–driven ERG response. The model fits to the threshold data allow the ratios of L-/M-cone weighting to be estimated, and they reveal phase differences between the L- and M-cone–driven ERG responses, which can be compared with the direct measurements.

For the majority of the normal subjects, the L-cone weighting was larger than the M-cone weighting, although in one normal observer (US) the ratio of the L-/M-cone weighting was below unity. As reported previously,[14] there is a considerable interindividual variability of the L-/M-cone–weighting ratio. This interindividual variability is reflected by the different orientations of the ellipses. The larger the L-/M-cone–weighting ratio the more the threshold ellipses are tilted toward the M-cone axis. This variability can be correlated with variations in the L-/M-cone–weighting ratios in the psychophysically assessed luminance channel and probably with the amount of

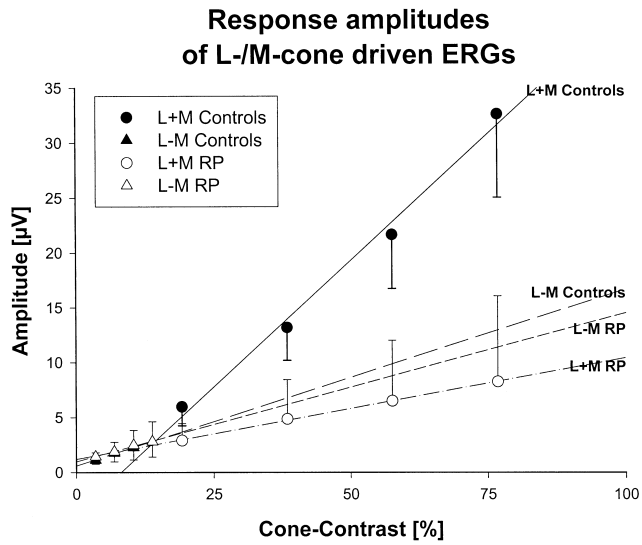

FIGURE 2. ERG response amplitudes as a function of cone contrast in the normal subjects (*filled symbols*: mean ± SD) and the RP patients (*open symbols*: mean ± SD) for modulation of the L- and M-cones in-phase (L + M; L–M cone contrast ratio, 1:1; *circles*) and in counter-phase (L − M, L–M cone contrast ratio, 1:−1; *triangles*). In accordance with previous data (see Fig. 3 in Usui et al.[16]), we found an approximately linear relationship between ERG-response amplitude and cone contrast for all conditions in both the RP patients and the normal subjects. The slope of the fitted line to the data points (obtained by linear regression) is the contrast gain of the ERG signal. The contrast gain of the normal subjects was larger when modulating the L- and M-cones in-phase than when modulating them in counter-phase, indicating an additive interaction. For the RP patients, the reverse was true, indicating subtractive interaction between the L-cone– and M-cone–driven ERG signals.

cones in the retina.[20,21] However, the major axes of the ellipses of all normal subjects are oriented within the second and fourth quadrants, which is indicative of an additive interaction between the L- and M-cone–driven responses.

Figure 3B shows the ERG thresholds for six patients with different forms of RP. For the RP patients, the ellipse orientations are completely different from those of the controls. This was observed in all patients for whom a model could be obtained, with the exception of one female carrier of X-RP. For those patients, the estimated phase differences between the L- and M-cone–driven response were between 140° and 180°, indicative of a subtractive interaction between L- and M-cone–driven ERGs. Such subtractive interactions were in accordance with the preliminary conclusions derived from Figures 1 and 2. In 15 patients three or fewer thresholds could be measured, preventing a reliable model fit. In 8 of these patients, the responses to counter-phase modulation were significantly larger than the responses to in-phase modulation, suggesting that the cone responses interact subtractively. In the remaining 7 patients a definite statement on the cone response interaction was not possible. None of the patients showed any evidence of additive interactions.

Cone Weightings

In the 11 patients for whom we could get reliable model fits, the L- and M-cone weightings (A_L and A_M, respectively) were estimated and compared with those of the controls. The cone weighting data were statistically analyzed with an ANOVA with subsequent multiple comparisons (resulting in post-hoc test Bonferroni probability values) between subject groups and cone type. The ANOVA revealed that the cone weightings differed significantly in the groups defined by subject group and cone type ($P < 0.0001$; $F = 36.46$). The post-hoc tests revealed that the average A_L is significantly larger than the average A_M for both the normal subjects ($P < 0.001$) and the RP patients ($P < 0.01$). Furthermore, A_L in the RP patients was significantly reduced compared with the normal subjects ($P < 0.01$), whereas the reduction in A_M was not significant (Fig. 4A).

From the cone weightings we calculated their L-/M-cone–weighting ratios (Fig. 4B). To obtain a normal distribution we converted the L-/M-cone–weighting ratios into their logarithms. An unpaired *t*-test did not reveal a significant difference between the two subject groups, suggesting that RP causes an equal reduction in the two cone pathways.

Owing to the large interindividual variability of L- and M-cone weightings, neither of them can be directly used to quantify the overall loss in ERG sensitivity of individual patients. We, therefore, quantified the loss in sensitivity by determining the theoretically least threshold defined as the smallest possible distance of the fitted ellipse to the origin (for an example see subject MW in Fig. 3A). This smallest possible distance can be derived analytically from the model fits using the following formula:

$$k = \frac{A_L^2 - A_M^2 \pm \sqrt{A_M^4 + A_L^4 + 2 \cdot A_L^2 \cdot A_M^2 \cdot (2\,cos^2[P_L - P_M] - 1)}}{2 \cdot A_L \cdot A_M \cdot cos(P_L - P_M)} \quad (1)$$

This formula gives two values for k. One is the ratio between the L- and M-cone modulation at which the ellipse has the least distance to the origin. The other is the ratio at the largest distance. A_L and A_M are the cone weightings of the L- and M-cone–driven ERGs; $P_L - P_M$ is the phase difference between the L- and M-cone–driven ERG responses; the three parameters are obtained from the model fits to the threshold data. The reciprocal value of the least threshold was defined as the maximal L-/M-cone–driven ERG sensitivity (S_m).

For all patients, for whom reliable model fits were obtained, the difference between S_m and the reciprocal value of the smallest measured thresholds was relatively small. We therefore used the smallest of all measured thresholds to estimate S_m for those patients for whom no reliable model fits were feasible. The mean S_m of the RP patients [0.1513 ± 0.111 μV (% cone contrast)$^{-1}$] was significantly smaller ($P < 10^{-7}$; unpaired *t*-test) than that of the normal subjects [0.320 ± 0.072 μV (% cone contrast)$^{-1}$].

Phases of Cone-Driven ERGs

From the Fourier analysis on the ERG responses to the cone-isolating stimuli, it was possible to obtain the phases of the L- and M-cone–driven ERGs directly. As discussed previously,[19] the actual phases can differ by integer multiples of 360° from the phases obtained from the Fourier analysis. We estimated the most probable response phases of the controls from phases at other temporal frequencies (unpublished data, 1999) and from implicit times, which were reported to range between 25 and 30 msec for the 30-Hz-fERG.[22] The response phases of the

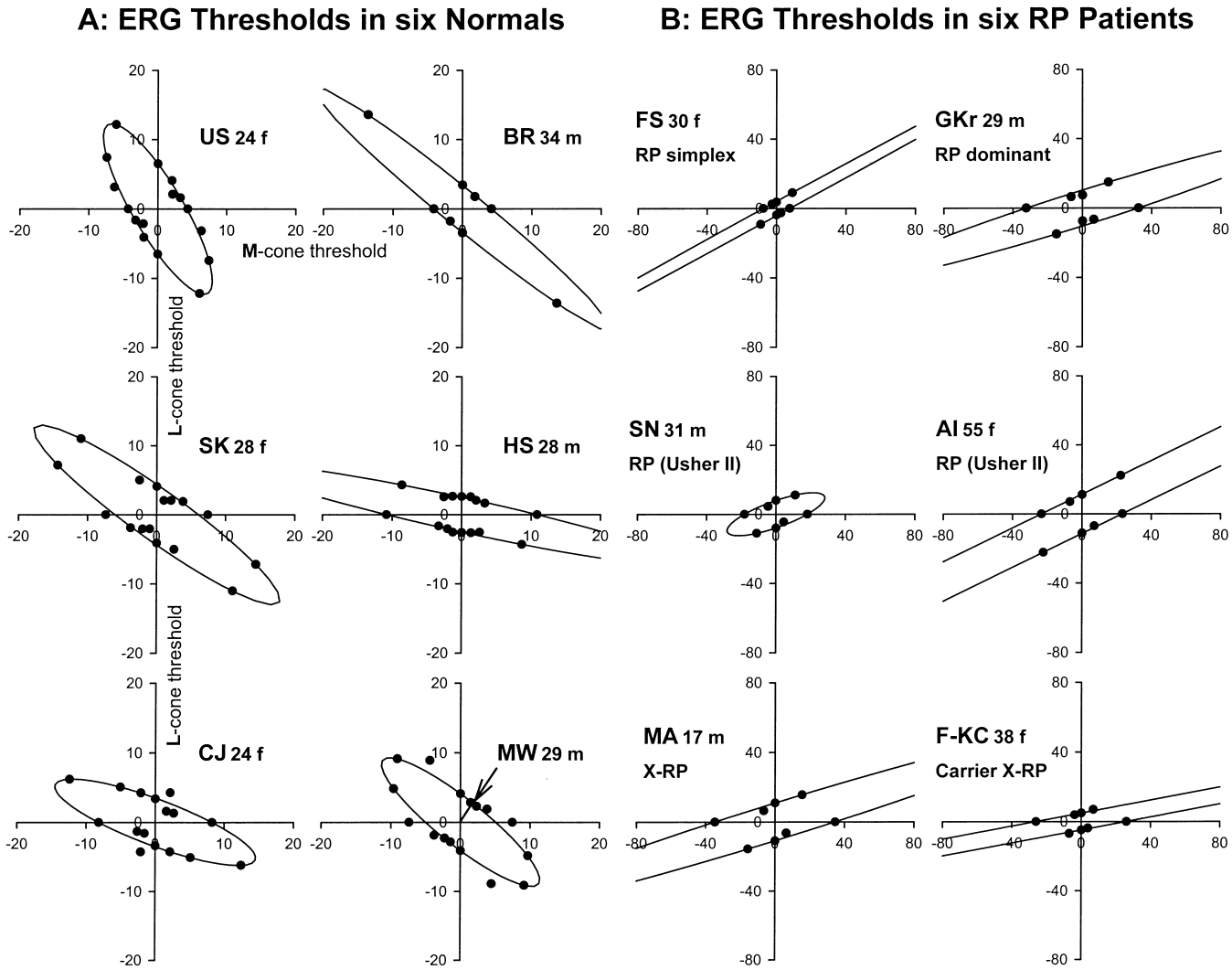

Figure 3. Threshold contrasts in six normal subjects (**A**, *left*) and six patients with different forms of RP (1 RP simplex, 1 autosomal recessive form, 2 Usher II syndrome, 1 male X-RP, and one female carrier of X-RP; **B**, *right*). The data points in the first and third quadrants and in the second and fourth quadrants are thresholds for physically identical stimulus conditions. The data points in the first and third quadrants are thresholds for stimulus conditions in which the L- and M-cones were modulated in-phase, whereas data points in the second and fourth quadrants indicate thresholds to counter-phase modulation of the two cone types. The ellipses are fits of a vector addition model to the data points. The *inset type* gives the initials of the subject, age and gender. There is a considerable interindividual variability of the L-/M-cone contrast gain ratio, resulting in different orientations of the fitted ellipses. In the normal observers, the major axes of all displayed ellipses are oriented within the second and fourth quadrants. In the RP patients, the major axes of the ellipses are oriented within the first and third quadrants. Note the different scaling for the normal subjects and the patients. RP patients generally exhibited larger thresholds compared with the normal observers, which corresponds to the overall reduction in sensitivity. For one normal subject (MW), a *line* (marked by an *arrow*) indicates the theoretically least threshold (the point on the ellipse with the smallest distance to the origin). The inverse of the least threshold is defined as the maximal sensitivity.

RP patients were assumed to be as close as possible to those of the controls. For the ensuing statistical analysis this was the worst-case scenario. The choice of the absolute value of the response phase had no influence on the interpretation of the data.

In Figure 5, the ERG response phases for the M- and L-cone–isolating stimuli are shown as a function of cone contrast. The phase data were only included when the response amplitudes were significantly above noise level (typically 0.3 μV). As has been observed previously,[19,23] the ERG response phase lag increased linearly with decreasing cone contrast for the controls within the range of cone contrasts used (but see Usui et al.[19] for the case that low cone contrasts are included).

We applied an ANCOVA to these phase data to correct for the influence of cone contrast. We assumed that the variability in the data were influenced by four factors: subject group (controls, RP patients), cone type, cone contrast, and subject number as a random effect. Furthermore, it was assumed that these factors could interact, that all measurement errors are identical, and that there is a linear relationship between response phase and cone contrast. Using the ANCOVA, we estimated four different straight lines describing the relationship between response phase and cone contrast for each subject group and each cone type satisfactorily (adj. $R^2 = 0.90$, residual SD 18°). In the normal subjects, the L- and M-cone–driven ERG response phase lags decreased significantly ($P < 0.0002$)

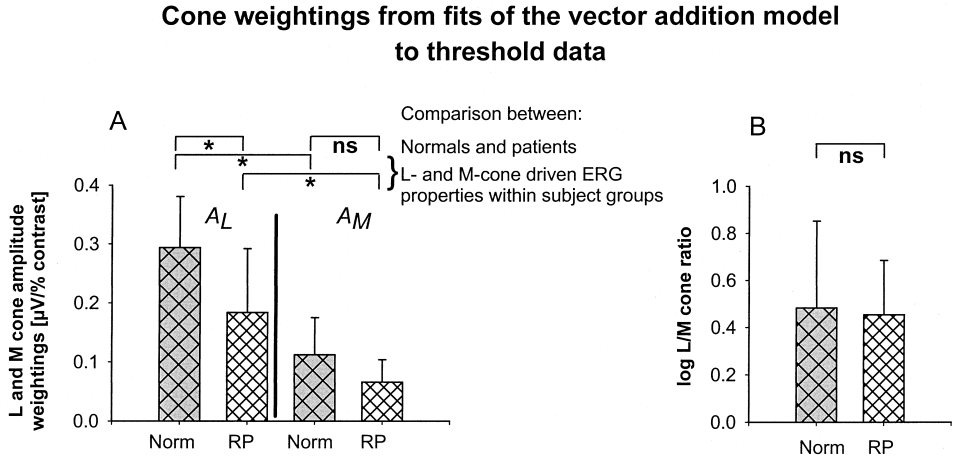

FIGURE 4. **(A)** Estimated L- and M-cone weightings (A_L and A_M: mean + SD) for the normal subjects and the RP patients derived from the fits of a vector addition model to the threshold data. The results of the ANOVA (post-hoc tests) on the weighting data are displayed above the histograms. **(B)** The L-/M-cone-weighting ratio given as log (A_L/A_M). The result of the *t*-test is displayed above the histograms. An *asterisk* indicates a significant difference; *ns* indicates a nonsignificant difference.

with increasing cone contrast (the slope was 1.6 ± 0.25°/% cone contrast [mean ± SE] for the L-cone–driven ERG phase and 1.2 ± 0.31°/% cone contrast for the M-cone–driven ERG phase). In the RP patients, the dependency of the response phase on cone contrast was variable between individual patients. As a result, the ANCOVA did not reveal a significant correlation between ERG phases and cone contrast in the patients as a group.

The mean estimated L-cone–driven ERG response phase (P_L) was −486° in the patients and −385° in the controls. Post-hoc tests revealed that P_L of the RP patients lagged the P_L of the controls significantly ($P < 0.001$). The mean estimated M-cone–driven ERG response phase (P_M) was −326° in the patients and −376° in the controls. Post-hoc tests revealed that P_M of the RP patients was significantly phase advanced compared with P_M of the controls ($P < 0.001$). Because the actual phase values can be integer multiples of 360° different from the phases calculated from the Fourier analysis, it cannot be excluded that P_M of the RP patients was −686° and, thus, lagged the normal responses extremely. However, this possibility seems very improbable. P_L and P_M differed significantly in the RP patients ($P < 0.001$) but not in the normal subjects. Previously, we concluded on the basis of largely the same data set that the difference between P_L and P_M in normal subjects was significant.[14] This seeming discrepancy is caused by the introduction of the patient data, which necessitated a correction for multiple comparisons in the present study. The uncorrected probability value indeed confirmed our previous conclusion. The mean phase data are summarized in Figure 6A.

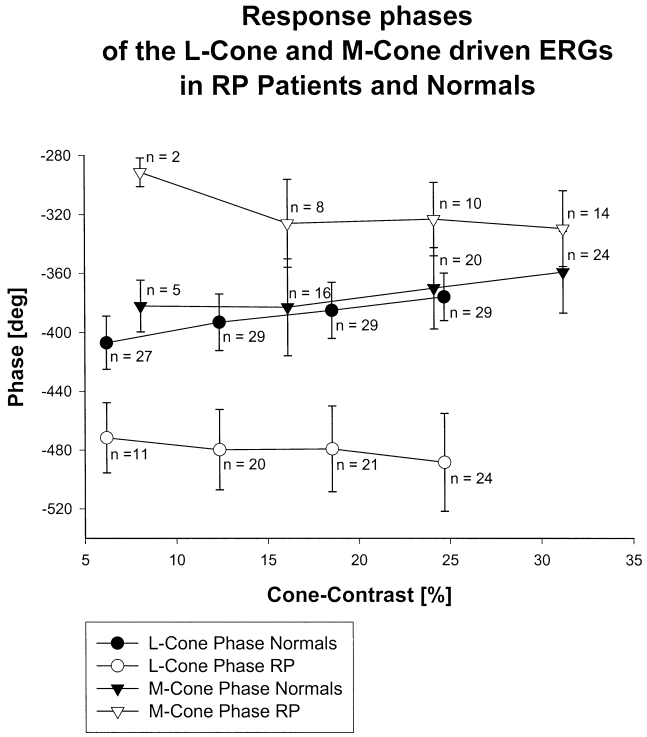

FIGURE 5. ERG response phase to cone-isolating stimuli as a function of cone contrast in normal subjects and RP patients (mean ± SD). The responses of the L-cone–driven ERGs are delayed in the RP patients compared with the normal subjects. The M-cone–driven ERGs are phase-advanced in the RP patients. The resultant phase difference between the ERG signals arising in the L- and M-cones is large in the RP patients.

Owing to the differential effect of RP on the phase lags of the L- and M-cone–driven ERGs, the mean phase difference of 160° (486–326°) was significantly larger than in the controls (9°; 385–376°). The phase differences imply a subtractive signal interaction between L- and M-cone–driven ERGs in the patients but an additive interaction in the controls.

Independent estimates of the phase differences between L- and M-cone–driven ERGs were available from the model fits to the threshold data. These phase differences are displayed in Figure 6B and differed significantly between patients and controls ($P < 10^{-10}$; unpaired *t*-test).

In Figure 7 the individual values of the two estimates of the phase differences are plotted against each other (only data points are shown for subjects in whom ERG thresholds to all different combinations of L- and M-cone modulation could be obtained), showing a positive correlation ($r = 0.94$). Obviously, the RP patients and the normal subjects fall into two separated groups. The phase differences estimated for one female carrier of X-RP were smaller than those of most other patients, but still larger than those of most controls.

DISCUSSION

Effects of RP on the L- and M-Cone–Driven ERGs

Our measurements reveal that the amplitudes of the L- and M-cone–driven ERGs are reduced in RP. However, the L-/M-

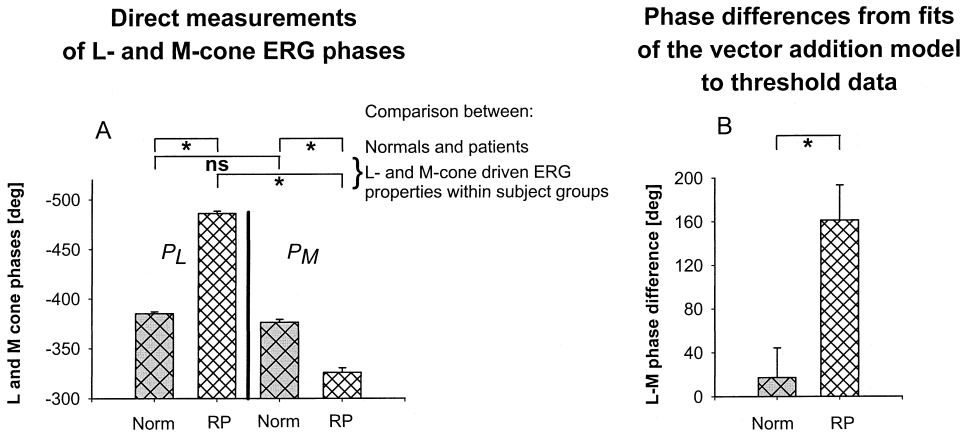

FIGURE 6. **(A)** Estimated phase data for the normal subjects and the RP patients derived from the direct measurements (mean + SE). After correction for the effect of cone contrast, the phase lag of the L-cone–driven ERG response of the patients is significantly increased, whereas the M-cone–driven ERG phase lag is significantly decreased. The results of the ANOVA (post-hoc test) are indicated above the histograms. **(B)** Phase differences between L- and M-cone–driven ERGs estimated from the fits of a vector addition model to the threshold data (mean + SD). The fit data revealed a highly significant increase in phase difference ($P < 10^{-10}$; unpaired *t*-test). This increase is in accordance with the changes in ERG response phases obtained from the direct measurements (*Asterisks* above the histograms indicate significant differences; *ns* indicates nonsignificant effects).

cone–weighting ratios are very similar, suggesting that the ERG sensitivities of the two cone pathways are equally reduced. The phase differences between the L- and M-cone–driven ERG signals are significantly increased. Despite the fact that the group of RP patients comprise different forms of inheritance and different phenotypes, all patients show consistently increased phase lags of the L-cone–driven ERG, and decreased phase lags of the M-cone–driven ERG compared with the normal subjects. Even patients who are relatively mildly affected (e.g., patient shown in Fig. 1B) exhibit large phase differences between the L- and M-cone–driven ERGs.

There is a tendency in the carriers of X-RP to have smaller phase changes in the L-cone–driven ERG and larger sensitivities. Furthermore, the carriers who show the smallest phase changes display the largest sensitivities. Thus, it seems that carriers might be generally less affected by RP, possibly caused by lyonization.[24]

Additionally, we found that in most RP patients, the response phase is negatively correlated with cone contrast. We previously observed a similar phase behavior in a patient with high myopia.[19] These types of anomalies in ERG phase can possibly be used as an extra diagnostic aspect.

In conclusion, the alterations of the interaction between the L- and M-cone–driven ERG signals are a sensitive indicator of RP, although it cannot be excluded that other retinal disturbances may lead to similar effects. Furthermore, our data indicate that this method allows some distinction between different forms of RP.

Implications for the Standard 30-Hz-fERG

In the 30-Hz-fERG, a white light source is luminance modulated[15] and will lead to an in-phase modulation of the L- and M-cones with approximately equal cone contrast. This condition is therefore comparable to the stimulus condition, in which the ratio of L- to M-cone contrasts is 1:1. (Of course, in contrast to the stimuli used in the present study, the 30-Hz fERG will also modulate the S-cones; but previous control measurements have shown that the S-cone contribution to the 30-Hz-fERG is negligible.[16]) We found that the largest sensitivity changes in RP occur in this condition. This is visualized in Fig. 8A, in which the threshold data of a normal subject (filled circles) and an RP patient (open squares) are shown, together with the model fits. Clearly, the threshold increase, and, thus, the loss in sensitivity, is extreme along the axis with the stimulus conditions equivalent to those used in the 30-Hz-fERG. The RP patients, who cooperated in the present study, show effectively reduced amplitudes of the 30-Hz-fERG (mean, 20 μV; SD, 19 μV; 5%–95% confidence interval, 47–112 μV). It, therefore, can be concluded that the amplitude of the 30-Hz-fERG is a sensitive tool to detect RP.

However, the 30-Hz-fERG ERG may lead to misinterpretations about the pathologic mechanisms that are responsible for the changes. Our data show that the loss of sensitivity along the axis approximating the 30-Hz-fERG conditions is mainly caused by an increased phase difference between the L- and M-cone–driven ERGs and to a lesser extent by a general sensitivity decrease. As can be expected from this, the amplitudes of the 30-Hz-fERG and the phase differences between the L- and M-cone–driven ERGs are negatively correlated ($r = -0.69$; $P = 0.02$). Some patients even display a marked increase in the phase difference without a decrease in S_m (e.g., the patient whose measurements are shown in Fig. 1B). These patients have substantially reduced response amplitudes in the 30-Hz-fERG. Five other patients for whom the 30-Hz-fERG remained below noise level still showed a significant response in at least one of our stimulus conditions.

Furthermore, the loss in response amplitude along the axis approximating the 30-Hz-fERG conditions will probably also depend strongly on the orientation of the elliptical threshold contours. This is visualized in Figure 8B for two patients with about equal S_m but with different orientations of the elliptical threshold contours, caused by differences in the L-/M-cone–

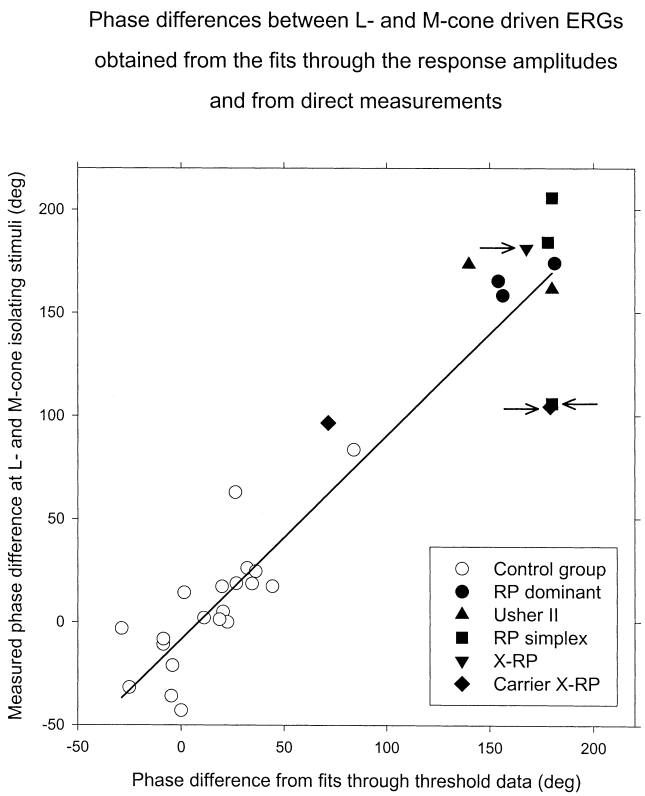

Figure 7. Phase differences between L- and M-cone–driven ERG responses obtained from the direct measurements as a function of the phase differences obtained from the model fits to the threshold data for normal subjects (*open symbols*) and RP patients (*filled symbols*; different symbols indicate subgroups within the population of the RP patients). Positive phase differences indicate that the M-cone–driven ERG responses were leading; negative phase differences indicate that the L-cone–driven ERG responses were leading (see also Fig. 5 in Kremers et al.[14]). The directly measured ERG response phases at 24.7% L-cone contrast and 23.4% M-cone contrast were used to calculate the differences. Owing to the similar cone contrasts, a possible confounding with the effect of cone contrast was minimized. For three data points (marked by the *arrows*) the measurements at the maximal possible M-cone contrast (31.2%) were used, because the responses at lower M-cone contrasts were below noise level. There is a clear positive correlation between the two values ($r = 0.94$). The linear regression [$f(x) = -8.4 + 0.99 * x$] through the data (*drawn line*) lies close to the expected diagonal. From this we conclude that both the fits and the direct measurements are reliable enough to quantify the phase difference in each subject.

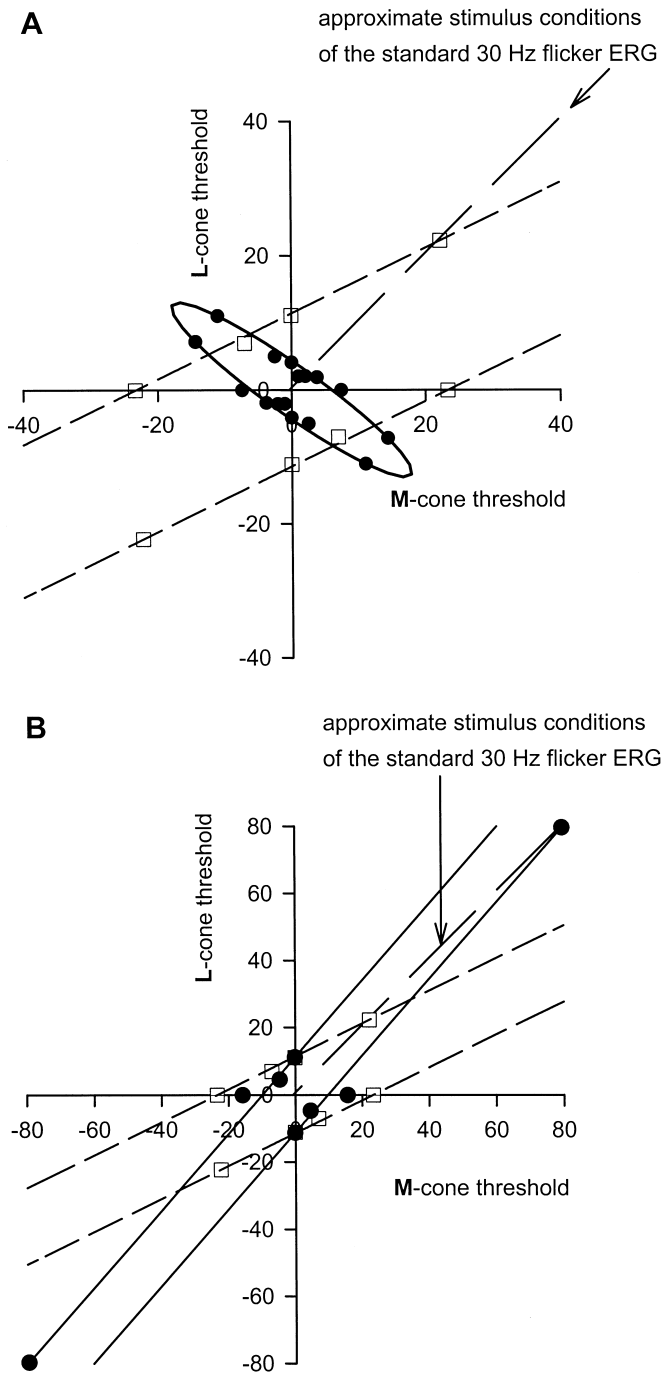

Figure 8. **(A)** Threshold contrasts in one normal subject (*filled circles*) and in one RP patient (*open squares*). The ellipses are fits of the vector addition model to the data points. The stimuli on the *long dashed line* under 45° closely resemble those used in the 30-Hz-fERG. For the RP patient, the threshold along this line is large, although relatively small thresholds were obtained at the other conditions. **(B)** Threshold contrasts in two RP patients with similar maximal sensitivities and similar phase differences between the L- and M-cone–driven ERGs but with different L-/M-cone–weighting ratios. The difference in L-/M-cone–weighting ratio is reflected by the different orientations of the fitted ellipses and leads to substantially different thresholds for the stimuli that are similar to those used in the 30-Hz-fERG.

weighting ratio.[14] The difference in the sensitivities between the two patients is about a factor of four for our stimulus condition approximating the 30-Hz-fERG (Fig. 8B) and about a factor of two in the directly measured 30-Hz-fERG.

Finally, an increase in the implicit time of the 30-Hz-fERG might be misinterpreted as a general feature of the cone-driven ERG. Our data show that only the L-cone–driven ERGs are delayed. The phase lags of the M-cone–driven ERGs are even decreased in the patients. The increase in implicit time in the 30-Hz-fERG is probably caused by the fact that the phase change of the L-cone–driven ERG is larger than of the M-cone–driven ERG and that the cone-driven ERGs of the majority of human subjects, including the RP patients, are dominated by the L-cones.[14,25]

Anatomic Substrates and Possible Mechanisms of the Phase Changes

The origin of the 30-Hz-fERG probably resides in the photoreceptors, the bipolar cells, and the Müller cells.[26,27] Therefore, the measured phase changes must be caused by alterations in at least one of these cell types. It is difficult to speculate on the mechanisms that are involved. It has been suggested that a reduction in the number of quantal catches in the photoreceptors of RP patients results in an increased phase lag.[4,28,29] This can only explain our data if the M-cone–driven ERGs of the patients lag those of the controls. As mentioned before, this is not very probable. Moreover, this explanation does not account for the different phase changes in the L- and M-cone–driven ERGs, unless there are separate changes in quantal absorption in the L- and the M-cones, either involving different reductions in the amounts of L- and M-cones or divergent changes in photopigment absorption spectra in individual cone outer segments (e.g., owing to gross changes in photopigment density). But, both mechanisms would also lead to a differential decrease in the L- and M-cone weightings. However, our data show that the L-/M-cone–weighting ratio is unaltered, suggesting an equal reduction in quantal catches in the two cone types. In addition, the calculations show that a decrease in the amount of cones or in photopigment density cannot result in counter-phase modulations of the L- and M-cones for all conditions in which they were modulated in-phase in the controls and, therefore, cannot lead to the subtractive interactions that we found in the RP patients.

It is commonly accepted that RP mainly affects the rod system. Because of the high retinal illuminance and the high temporal frequency used in this study, direct rod activity in the measured ERG signals can be excluded.[22] An extra indication that the rod signal is negligible comes from measurements in deuteranopes, in which we obtained small responses in the M-cone–isolating condition despite the large rod contrast that is present in this stimulus.[14,16] Furthermore, the rod responses in the standard ERG are either extinguished or substantially reduced in the RP patients, suggesting that their L- and M-cone–driven ERG responses do not originate in the rods. However, we cannot exclude the possibility that progressive rod degeneration changes the properties of the L- and M-cone systems or of postreceptoral additive and subtractive mechanisms in a different manner.

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